A 37

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 May 2003 (15.05.2003)

PCT

(10) International Publication Number
WO 03/039550 A1

(51) International Patent Classification[7]: A61K 31/506, A61P 17/00, 35/00, 43/00, A61K 7/48

(21) International Application Number: PCT/IB02/04330

(22) International Filing Date:
20 September 2002 (20.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/323,312 20 September 2001 (20.09.2001) US

(71) Applicant *(for all designated States except US)*: **AB SCIENCE** [FR/FR]; 3, avenue Georges V, F-75008 Paris (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MOUSSY, Alain** [FR/FR]; 22 bis, Passage Dauphine, F-75006 Paris (FR). **KINET, Jean-Pierre** [FR/US]; 3 Hunt Road, Lexington, MA 02421 (US).

(74) Agents: **MARTIN, Jean-Jacques** et al.; Cabinet Regimbeau, 20, Rue de Chazelles, F-75847 Paris Cedex 17 (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
— *of inventorship (Rule 4.17(iv)) for US only*
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/039550 A1

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR WHITENING HUMAN SKIN AND TREATING MELANOCYTE DYSFUNCTION ASSOCIATED DISEASES

(57) Abstract: The present invention relates to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Use of tyrosine kinase inhibitors for whitening human skin and treating melanocyte dysfunction associated diseases

The present invention relates to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The skin consists of two layers, the epidermis and the underlying dermis, which are separated by the basal membrane. Melanocytes are found in the basal layer and exhibit generally globular cellular morphologies with numerous dendritic ramifications. These highly specialized cells penetrate the neighboring keratinocytes of the basal layer. Melanocytes have melanosomes, which produces the melanin pigments. Melanosomes are released from the melanocyte dendrites onto the surroundings of neighboring cells, thereby diffusing pigmentation across the skin.

Melanosomes produces melanin thanks to tyrosinase, which catalyzes the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-Dopa), which is a melanin precursor. The melanosomes migrate from the Golgi apparatus through the cell body and into the dendrites, in the process accumulating melanin. At last, melanin serves as a natural solar filter, absorbing over 90% of the UV radiation passing through the horny layer of skin, thereby protecting DNA from UV-induced modification.

Skin pigmentation is directly proportional to the quantity of melanocytes in the skin. Hyperpigmentation patterns can reflect concentrations of correctly proliferating melanocytes, or the results of improper proliferation. The former category of hyper pigmentation include freckles, chloasma (a hypersecretion of melanin induced by hormonal factors and amplified by the effects of the sun), and various forms of hypermelanosis. Other excessive skin pigmentation resulting from melanocyte dysfunction include lentigines, solar and senile lentigo, Dubreuilh melanosis (a precancerous condition), moles and malignant melanomas.

In Asia and other parts of the world, women may desire whiter skin because of traditional beliefs that white skin denotes nobility and beauty. Thus, in recent years, cosmetic compositions have been developed to reduce the amount of melanin in the skin and therefore, whiten the skin.

As a result, there is real need in the development of cosmetic and clinical treatments for whitening the skin.

In the past, chemicals used to whiten skin, such as hydrogen peroxide, mercurialized amide chlorate, mercaptoamines and phenol derivatives such as hydroquinone irritate the skin. Modern compositions are more selective in their effects and better tolerated. Indeed, research has focused on whitening agents that inhibit the activity of tyrosinase, which plays a key role in the biosynthesis of melanin. For example, it has been proposed to incorporate into cosmetic compositions tyrosinase inhibitors such as hydroquinone, vitamin C and its derivatives, kojic acid, arbutin, glutathione, cysteine as well as plant extracts (US 5,773,014 and US 5,980,904).

However, tyrosinase inhibitors such as kojic acid, ascorbic acid and their derivatives are unstable in cosmetic preparations and their rapid oxidation decreases tyrosinase inhibition and results in the black coloration of the preparations. Moreover, although less cytotoxic than phenol derivatives, tyrosinase inhibitors exhibit unwanted side effects.

As a result, alternative solutions are required for whitening human skin without causing irritation and toxicity to the epidermis and underlying dermis. Advantageously, it is of special interest to provide a composition which is effective for the treatment of melanocyte dysfunction related diseases leading to hyperpigmentation.

Grichnik et al, J Invest Dermatol 1998 Aug;111(2):233-8 have demonstrated that the SCF/KIT pathway is implicated in the control of normal human melanocyte homeostasis. On histologic evaluation, SCF injection increased the number, size, and dendricity of melanocytes.

In connection with the present invention, it is proposed to use specific kinase inhibitors to inhibit the SCF/KIT pathway which is responsible for melanocytes proliferation. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited to reach this goal. Furthermore, activating mutations in the c-kit receptor is postulated to induce abnormal proliferation of melanocytes leading to the formation of melanomas.

Such inhibitors are not only useful for whitening the skin but they are good candidate for treating hypermelanosis resulting from melanocyte dysfunction and including lentigines, solar and senile lentigo, Dubreuilh melanosis, moles as well as malignant melanomas.

Description

The present invention relates to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.

Tyrosine kinase inhibitors are selected for example from bis monocyclic, bicyclic or heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO 94/14808) and 1-cycloproppyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504, US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940) and aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

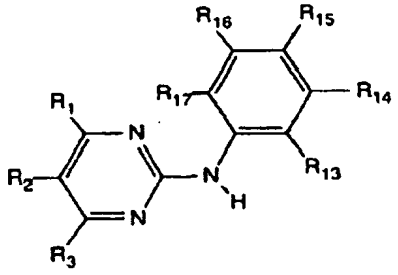
Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

In another embodiment, the invention is directed to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering a c-kit inhibitor to a human in need of such treatment.

Preferably, said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor. Such inhibitors can be selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

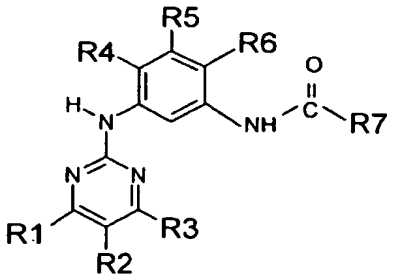
Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940), 4-amino-substituted quinazolines (US 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (US 3,800,039), aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (US 4,464,375), and 4-thienyl-2-(1H)-quinazolones (US 3,551,427).

So, preferably, the invention relates to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering a non toxic, potent and selective c-kit inhibitor which is a pyrimidine derivative, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I :



wherein the R1, R2, R3, R13 to R17 groups have the meanings depicted in EP 564 409 B1, incorporated herein in the description.

Preferably, the N-phenyl-2-pyrimidine-amine derivative is selected from the compounds corresponding to formula II :

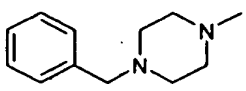


Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function.

Preferably, R7 is the following group :



Among these compounds, the preferred are defined as follows:

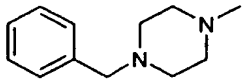
R1 is a heterocyclic group, especially a pyridyl group,

R2 and R3 are H,

R4 is a C1-C3 alkyl, especially a methyl group,

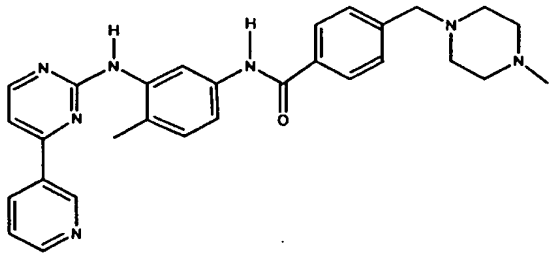
R5 and R6 are H,

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, for example the group :



Therefore, in a preferred embodiment, the invention relates to a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising the administration of an effective amount of the compound known in the art as CGP57148B :

4-(4-méhylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino)phényl]-benzamide corresponding to the following formula :



The preparation of this compound is described in example 21 of EP 564 409 and the β-form, which is particularly useful is described in WO 99/03854.

Alternatively, the c-kit inhibitor can be selected from :

- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phenyl-6,7-dimethoxy quinaxoline.

In a preferred aspect, the invention contemplated the method mentioned above, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

In a further embodiment, c-kit inhibitors as mentioned above are inhibitors of activated c-kit. In frame with the invention, the expression "activated c-kit" means a constitutively activated-mutant c-kit including at least one mutation selected from point mutations, deletions, insertions, but also modifications and alterations of the natural c-kit sequence (SEQ ID N°1). Such mutations, deletions, insertions, modifications and alterations can occur in the transphosphorylase domain, in the juxtamembrane domain as well as in any domain directly or indirectly responsible for c-kit activity. The expression "activated c-kit" also means herein SCF-activated c-kit. Preferred and optimal SCF concentrations for activating c-kit are comprised between 5.10^{-7} M and 5.10^{-6} M, preferably around 2.10^{-6} M. In a preferred embodiment, the activated-mutant c-kit in step a) has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants. In another preferred embodiment, the activated-mutant c-kit in step a) has a deletion in the juxtamembrane domain of c-kit. Such a deletion is for example between codon 573 and 579 called c-kit d(573-579). The point mutation V559G proximal to the juxtamembrane domain c-kit is also of interest.

In this regard, the invention contemplates a method for whitening human skin and treating melanocyte dysfunction associated diseases comprising administering to a

mammal in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,

b) selecting compounds that inhibit activated c-kit,

c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

This screening method can further comprise the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit (for example in the transphosphorylase domain), which are also capable of inhibiting SCF-activated c-kit wild.

Alternatively, in step a) activated c-kit is SCF-activated c-kit wild.

A best mode for practicing this method consists of testing putative inhibitors at a concentration above 10 μM in step a). Relevant concentrations are for example 10, 15, 20, 25, 30, 35 or 40 μM.

In step c), IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

Examples of IL-3 dependent cells include but are not limited to :

- cell lines naturally expressing and depending on c-kit for growth and survival. Among such cells, human mast cell lines can be established using the following procedures : normal human mast cells can be infected by retroviral vectors containing sequences coding for a mutant c-kit comprising the c-kit signal peptide and a TAG sequence allowing to differentiate mutant c-kits from c-kit wild expressed in hematopoetic cells by means of antibodies.

This technique is advantageous because it does not induce cellular mortality and the genetic transfer is stable and gives satisfactory yields (around 20 %). Pure normal human mast cells can be routinely obtained by culturing precursor cells originating from blood obtained from human umbilical vein. In this regard, heparinated blood from umbilical vein is centrifuged on a Ficoll gradient so as to isolate mononucleated cells from other blood components. CD34+ precursor cells are then purified from the isolated cells mentioned above using the immunomagnetic selection system MACS (Miltenyi biotech). CD34+ cells are then cultured at 37°C in 5 % CO_2 atmosphere at a concentration of 10^5 cells per ml in the medium MCCM (α-MEM supplemented with L-glutamine, penicillin, streptomycin, 5 10^{-5} M β-mercaptoethanol, 20 % veal fœtal serum, 1 % bovine albumin serum and 100 ng/ml recombinant human SCF. The medium is changed every 5 to 7 days. The percentage of mast cells present in the culture is assessed each week, using May-Grünwal Giemsa or Toluidine blue coloration. Anti-tryptase antibodies can also be used to detect mast cells in culture. After 10 weeks of culture, a pure cellular population of mast cells (> 98 %) is obtained.

It is possible using standard procedures to prepare vectors expressing c-kit for transfecting the cell lines established as mentioned above. The cDNA of human c-kit has been described in Yarden et al., (1987) EMBO J.6 (11), 3341-3351. The coding part of c-kit (3000 bp) can be amplified by PCR and cloned, using the following oligonucleotides :

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No2) sens
- 5'CTGCTTCGCGGCCGCGTTAACTCTTCTCAACCA3' (SEQ ID No3) antisens

The PCR products, digested with NotI and XhoI, has been inserted using T4 ligase in the pFlag-CMV vector (SIGMA), which vector is digested with NotI and XhoI and

dephosphorylated using CIP (Biolabs). The pFlag-CMV-c-kit is used to transform bacterial clone XL1-blue. The transformation of clones is verified using the following primers :

- 5'AGCTCGTTTAGTGAACCGTC3' (SEQ ID No4) sens,
- 5'GTCAGACAAAATGATGCAAC3' (SEQ ID No5) antisens.

Directed mutagenesis is performed using relevant cassettes is performed with routine and common procedure known in the art..

The vector Migr-1 (ABC) can be used as a basis for constructing retroviral vectors used for transfecting mature mast cells. This vector is advantageous because it contains the sequence coding for GFP at the 3' and of an IRES. These features allow to select cells infected by the retrovirus using direct analysis with a fluorocytometer. As mentioned above, the N-terminal sequence of c-kit c-DNA can be modified so as to introduce a Flag sequence that will be useful to discriminating heterogeneous from endogenous c-kit.

Other IL-3 dependent cell lines that can be used include but are not limited to:

- BaF3 mouse cells expressing wild-type or mutated form of c-kit (in the juxtamembrane and in the catalytic sites) are described in Kitayama et al, (1996), Blood 88, 995-1004 and Tsujimura et al, (1999), Blood 93, 1319-1329.
- IC-2 mouse cells expressing either c-kit^{WT} or c-kit^{D814Y} are presented in Piao et al, (1996), Proc. Natl. Acad. Sci. USA 93, 14665-14669.

IL-3 independent cell lines are :

- HMC-1, a factor-independent cell line derived from a patient with mast cell leukemia, expresses a juxtamembrane mutant c-kit polypeptide that has constitutive kinase activity (Furitsu T et al, J Clin Invest. 1993;92:1736-1744 ; Butterfield et al, Establishment of an

immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345-355 and Nagata et al, Proc Natl Acad Sci U S A. 1995;92:10560-10564).

- P815 cell line (mastocytoma naturally expressing c-kit mutation at the 814 position) has been described in Tsujimura et al, (1994), Blood 83, 2619-2626.

The extent to which component (ii) inhibits activated c-kit can be measured *in vitro* or *in vivo*. In case it is measured *in vivo*, cell lines expressing an activated-mutant c-kit, which has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants, are preferred.
Example of cell lines expressing an activated-mutant c-kit are as mentioned.

In another preferred embodiment, the method further comprises the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μM. This can be measured *in vitro* or *in vivo*.

Therefore, compounds are identified and selected according to the method described above are potent, selective and non-toxic c-kit wild inhibitors.

Alternatively, the screening method as defined above can be practiced *in vitro*. In this regard, the inhibition of mutant-activated c-kit and/or c-kit wild can be measured using standard biochemical techniques such as immunoprecipitation and western blot. Preferably, the amount of c-kit phosphorylation is measured.

In a still further embodiment, the invention contemplates a method for whitening human skin and treating melanocyte dysfunction associated diseases as depicted above wherein the screening comprises :

a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC50 < 10 μM, by measuring the extent of cell death,

b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,

c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC50 < 10 μM, preferably an IC50 < 1 μM, by measuring the extent of cell death.

Here, the extent of cell death can be measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide. These are common techniques routinely practiced in the art.

The expression "melanocyte dysfunction associated diseases" will be understood herein as hypermelanosis resulting from melanocyte dysfunction and including lentigines, solar and senile lentigo, Dubreuilh melanosis, moles as well as melanomas including malignant melanomas.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament or a cosmetic composition for whitening human skin and treating melanocyte dysfunction associated diseases as defined above.

The pharmaceutical or cosmetic compositions utilized in this invention may be administered by any number of routes including oral and topical.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical compositions suitable for use in the invention include compositions wherein c-kit inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. As mentioned above, a tyrosine kinase inhibitor and more particularly a c-kit inhibitor according to the invention is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Preferably, the method according to the invention consists of applying to the skin a composition comprising an effective amount of a tyrosine kinase inhibitor as depicted above and a carrier acceptable for external use.

Thus, the invention also concerns a pharmaceutical or cosmetic composition for topical administration comprising a tyrosine kinase inhibitor and optionally at least one compound selected from the group consisting of tyrosinase inhibitors as mentioned above.

The compositions according to the invention may be presented in all forms normally used for topical application, in particular in the form of a gel, paste, ointment, cream, lotion, liquid suspension aqueous, aqueous-alcoholic or, oily solutions, or dispersions of the lotion or serum type, or anhydrous or lipophilic gels, or emulsions of liquid or semi-solid consistency of the milk type, obtained by dispersing a fatty phase in an aqueous phase or vice versa, or of suspensions or emulsions of soft, semi-solid consistency of the cream or gel type, or alternatively of microemulsions, of microcapsules, of microparticles or of vesicular dispersions to the ionic and/or nonionic type. These compositions are prepared according to standard methods.

The composition according to the invention comprises any ingredient commonly used in dermatology and cosmetic. It may comprise at least one ingredient selected from hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preservatives, emollients, viscosity enhancing polymers, humectants, surfactants, preservatives, antioxidants, solvents, and fillers, antioxidants, solvents, perfumes, fillers, screening agents, bactericides, odor absorbers and coloring matter.

As oils which can be used in the invention, mineral oils (liquid paraffin), vegetable oils (liquid fraction of shea butter, sunflower oil), animal oils, synthetic oils, silicone oils

(cyclomethicone) and fluorinated oils may be mentioned. Fatty alcohols, fatty acids (stearic acid) and waxes (paraffin, carnauba, beeswax) may also be used as fatty substances.

As emulsifiers which can be used in the invention, glycerol stearate, polysorbate 60 and the PEG-6/PEG-32/glycol stearate mixture are contemplated.

As hydrophilic gelling agents, carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, polysaccharides such as hydroxypropylcellulose, clays and natural gums may be mentioned, and as lipophilic gelling agents, modified clays such as bentones, metal salts of fatty acids such as aluminum stearates and hydrophobic silica, or alternatively ethylcellulose and polyethylene may be mentioned.

As hydrophilic active agents, proteins or protein hydrolysates, amino acids, polyols, urea, allantoin, sugars and sugar derivatives, vitamins, starch and plant extracts, in particular those of Aloe vera may be used.

As lipophilic active, agents, retinol (vitamin A) and its derivatives, tocopherol (vitamin E) and its derivatives, essential fatty acids, ceramides and essential oils may be used. These agents add extra moisturizing or skin softening features when utilized.

If desired, a known gelling agent may be added to the composition of the invention. Suitable gelling agents include a synthetic high molecular weight crosslinked polymer of acrylic acid, more specifically an acrylate/C.sub.10-30 alkyl acrylate copolymer available for example under the trade name CARBOMER 1342. Other suitable gelling agents include cellulose and cellulose derivatives such as dihydroxyethyl cellulose (tradename ULTRAGEL).

In addition, a surfactant can be included in the composition so as to provide deeper penetration of the ingredients and of the tyrosine kinase inhibitor.

Among the contemplated ingredients, the invention embraces penetration enhancing agents selected for example from the group consisting of mineral oil, water, ethanol, triacetin, glycerin and propylene glycol; cohesion agents selected for example from the group consisting of polyisobutylene, polyvinyl acetate and polyvinyl alcohol, and thickening agents.

Chemical methods of enhancing topical absorption of drugs are well known in the art. For example, compounds with penetration enhancing properties include sodium lauryl sulfate (Dugard, P. H. and Sheuplein, R. J., "Effects of Ionic Surfactants on the Permeability of Human Epidermis: An Electrometric Study," J. Ivest. Dermatol., V.60, pp. 263-69, 1973), lauryl amine oxide (Johnson et. al., US 4,411,893), azone (Rajadhyaksha, US 4,405,616 and 3,989,816) and decylmethyl sulfoxide (Sekura, D. L. and Scala, J., "The Percutaneous Absorption of Alkylmethyl Sulfides," Pharmacology of the Skin, Advances In Biolocy of Skin, (Appleton-Century Craft) V. 12, pp. 257-69, 1972). It has been observed that increasing the polarity of the head group in amphoteric molecules increases their penetration-enhancing properties but at the expense of increasing their skin irritating properties (Cooper, E. R. and Berner, B., "Interaction of Surfactants with Epidermal Tissues: Physiochemical Aspects," Surfactant Science Series, V. 16, Reiger, M. M. ed. (Marcel Dekker, Inc.) pp. 195-210, 1987).

Suitable solvents include alkyl esters of fatty acids, preferably C.sub.1-12, more preferably C.sub.3-10, alkyl esters of saturated or unsaturated fatty acids containing 8-22 carbon atoms. Particularly preferred solvents include isopropyl myristate, octyl palmitate, WIKENOL 161 (a mixture of esters), etc. Alcohols such as ethanol, propanol,

isopropanol, propylene glycol, etc., as well as aqueous mixtures of these alcohols may also be used.

A second class of chemical enhancers are generally referred to as co-solvents. These materials are absorbed topically relatively easily, and, by a variety of mechanisms, achieve permeation enhancement for some drugs. Ethanol (Gale et. al., U.S. Pat. No. 4,615,699 and Campbell et. al., U.S. Pat. Nos. 4,460,372 and 4,379,454), dimethyl sulfoxide (US 3,740,420 and 3,743,727, and US 4,575,515), and glycerine derivatives (US 4,322,433) are a few examples of compounds which have shown an ability to enhance the absorption of various compounds.

The invention is aimed at a composition which is formulated for the delivery of the tyrosine kinase inhibitor to the skin whether it is a cosmetic or dermatologic composition.

CLAIMS

1. A method for whitening human skin and treating melanocyte dysfunction associated diseases, comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.

2. A method according to claim 1, wherein said tyrosine kinase inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

3. A method for whitening human skin and treating melanocyte dysfunction associated diseases, comprising administering a c-kit inhibitor to a human in need of such treatment.

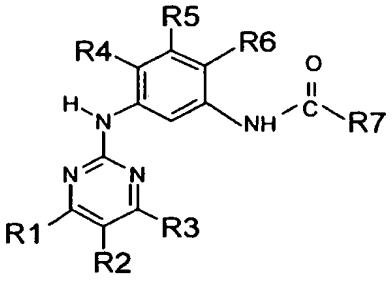
4. A method according to claim 3, wherein said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor.

5. A method according to claim 4, wherein said inhibitor is selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

6. A method according to claim 4, wherein said inhibitor is selected from the group consisting of :

- pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives.
- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds,
- and quinazoline derivatives.

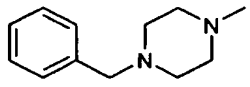
7. A method according to claim 4, wherein said inhibitor is selected from the group consisting of N-phenyl-2-pyrimidine-amine derivatives having the formula II :



Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, preferably the following group :



8. A method according to claim 7, wherein said inhibitor is the 4-(4-méhylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino)phényl]-benzamide.

9. A method according to one of claims 3 to 6, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

10. A method according to one of claims 3 to 9, wherein said c-kit inhibitor is an inhibitor of activated c-kit.

11. A method according to one of claims 3 to 9, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

12. A method according to claim 10, wherein said inhibitor is capable of inhibiting constitutively activated-mutant c-kit.

13. A method for whitening human skin and treating melanocyte dysfunction associated diseases, comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,

b) selecting compounds that inhibit activated c-kit,

c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

14. A method according to claim 13, wherein the screening method further comprises the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit, which are also capable of inhibiting SCF-activated c-kit wild.

WO 03/039550 PCT/IB02/04330

15. A method according to claim 13, wherein activated c-kit is SCF-activated c-kit wild in step a).

16. A method according to one of claims 13 to 15, wherein putative inhibitors are tested at a concentration above 10 μM in step a).

17. A method according to one of claims 13 to 16, wherein IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

18. A method according to one of claims 13 to 16, wherein IL-3 dependent cells are selected from the group consisting of mast cells, transfected mast cells, BaF3, and IC-2.

19. A method according to one of claims 13 to 18, wherein the extent to which component (ii) inhibits activated c-kit is measured *in vitro* or *in vivo*.

20. A method according to one of claims 13 to 19, further comprising the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μM.

21. A method according to claim 20, wherein the testing is performed *in vitro* or *in vivo*.

22. A method according to one of claims 13 to 21, wherein the inhibition of mutant-activated c-kit and/or c-kit wild is measured using standard biochemical techniques such as immunoprecipitation and western blot.

23. A method according to one of claims 13 to 22, wherein the amount of c-kit phosphorylation is measured.

24. A method according to one of claims 13 to 23, wherein identified and selected compounds are potent, selective and non-toxic c-kit wild inhibitors.

25. A method for whitening human skin and treating melanocyte dysfunction associated diseases, comprising administering to a human in need of such treatment a c-kit inhibitor obtainable by a screening method comprising :

a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC50 < 10 μM, by measuring the extent of cell death,

b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,

c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC50 < 10 μM, preferably an IC50 < 1 μM, by measuring the extent of cell death.

26. A method according to claim 25, wherein the extent of cell death is measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide.

27. A method according to one of claims 1 to 26 for whitening human skin and treating melanocyte dysfunction associated diseases, including hypermelanosis resulting from

melanocyte dysfunction such as lentigines, solar and senile lentigo, Dubreuilh melanosis, moles as well as melanomas, including malignant melanomas.

28. Use of a c-kit inhibitor to manufacture a medicament or a cosmetic composition for whitening human skin and treating melanocyte dysfunction associated diseases, including hypermelanosis resulting from melanocyte dysfunction such as lentigines, solar and senile lentigo, Dubreuilh melanosis, moles as well as malignant melanomas.

29. A composition suitable for oral or topical administration comprising a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for whitening human skin and treating melanocyte dysfunction associated diseases, including hypermelanosis resulting from melanocyte dysfunction such as lentigines, solar and senile lentigo, Dubreuilh melanosis, moles as well as malignant melanomas.

30. A pharmaceutical or cosmetic composition according to claim 29, which is suitable for topical application.

31. A composition according to claim 30, which is in the form of a gel, paste, ointment, cream, lotion, liquid suspension aqueous, aqueous-alcoholic or, oily solutions, or dispersions of the lotion or serum type, or anhydrous or lipophilic gels, or emulsions of liquid or semi-solid consistency of the milk type, obtained by dispersing a fatty phase in an aqueous phase or vice versa, or of suspensions or emulsions of soft, semi-solid consistency of the cream or gel type, or alternatively of microemulsions, of microcapsules, of microparticles or of vesicular dispersions to the ionic and/or nonionic type.

32. A composition according to claim 30, which comprises at least one ingredient selected from hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active

agents, emollients, viscosity enhancing polymers, humectants, surfactants, preservatives, antioxidants, solvents, and fillers.

33. A composition according to claim 30, which is formulated for the delivery of the tyrosine kinase inhibitor to the skin.

SEQUENCE LISTING

<110> AB Science

<120> Use of tyrosine kinase inhibitors for whitening human skin and treating melanocyte dysfunction associated diseases

<130> D19833 NT

<150> US 60/323,312
<151> 2001-09-20

<160> 5

<170> PatentIn Ver. 2.1

<210> 1
<211> 976
<212> PRT
<213> Homo sapiens

<220>
<223> Human c-kit

<400> 1

```
Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu
  1               5                  10                  15

Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly
             20                  25                  30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val
         35                  40                  45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
     50                  55                  60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
 65                  70                  75                  80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr
                 85                  90                  95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg
            100                 105                 110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
        115                 120                 125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
    130                 135                 140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu
145                 150                 155                 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
                165                 170                 175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly
            180                 185                 190

Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe
        195                 200                 205

Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
    210                 215                 220
```

Glu Gly Glu Glu Phe Thr Val Thr Cys Thr Ile Lys Asp Val Ser Ser
225 230 235 240

Ser Val Tyr Ser Thr Trp Lys Arg Glu Asn Ser Gln Thr Lys Leu Gln
245 250 255

Glu Lys Tyr Asn Ser Trp His His Gly Asp Phe Asn Tyr Glu Arg Gln
260 265 270

Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
275 280 285

Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser Ala Asn Val Thr Thr Thr
290 295 300

Leu Glu Val Val Asp Lys Gly Phe Ile Asn Ile Phe Pro Met Ile Asn
305 310 315 320

Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
325 330 335

Tyr Glu Ala Phe Pro Lys Pro Glu His Gln Gln Trp Ile Tyr Met Asn
340 345 350

Arg Thr Phe Thr Asp Lys Trp Glu Asp Tyr Pro Lys Ser Glu Asn Glu
355 360 365

Ser Asn Ile Arg Tyr Val Ser Glu Leu His Leu Thr Arg Leu Lys Gly
370 375 380

Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val Ser Asn Ser Asp Val Asn
385 390 395 400

Ala Ala Ile Ala Phe Asn Val Tyr Val Asn Thr Lys Pro Glu Ile Leu
405 410 415

Thr Tyr Asp Arg Leu Val Asn Gly Met Leu Gln Cys Val Ala Ala Gly
420 425 430

Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
435 440 445

Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
450 455 460

Ser Gly Pro Pro Phe Gly Lys Leu Val Val Gln Ser Ser Ile Asp Ser
465 470 475 480

Ser Ala Phe Lys His Asn Gly Thr Val Glu Cys Lys Ala Tyr Asn Asp
485 490 495

Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe Ala Phe Lys Gly Asn Asn
500 505 510

Lys Glu Gln Ile His Pro His Thr Leu Phe Thr Pro Leu Leu Ile Gly
515 520 525

Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr
530 535 540

Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val
545 550 555 560

Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr Ile Asp Pro Thr Gln Leu
565 570 575

```
Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly
            580                 585                 590

Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
        595                 600                 605

Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
    610                 615                 620

Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu
625                 630                 635                 640

Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu
                645                 650                 655

Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr
            660                 665                 670

Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
        675                 680                 685

Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
    690                 695                 700

Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu
705                 710                 715                 720

Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
                725                 730                 735

Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val
            740                 745                 750

Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
        755                 760                 765

Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala
    770                 775                 780

Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
785                 790                 795                 800

Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp
                805                 810                 815

Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
            820                 825                 830

Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
        835                 840                 845

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
    850                 855                 860

Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
865                 870                 875                 880

Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
                885                 890                 895

Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
            900                 905                 910

Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
        915                 920                 925
```

Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
930 935 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
945 950 955 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
965 970 975

<210> 2
<211> 30
<212> DNA
<213> Homo sapiens

<220>
<223> Primer

<400> 2
aagaagagat ggtacctcga ggggtgaccc 30

<210> 3
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Primer

<400> 3
ctgcttcgcg gccgcgttaa ctcttctcaa cca 33

<210> 4
<211> 20
<212> DNA
<213> Homo sapiens

<220>
<223> Primer

<400> 4
agctcgttta gtgaaccgtc 20

<210> 5
<211> 20
<212> DNA
<213> Homo sapiens

<220>
<223> Primer
<400> 5
gtcagacaaa atgatgcaac 20

INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/IB 02/04330

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/506 A61P17/00 A61P35/00 A61P43/00 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, MEDLINE, CHEM ABS Data, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRICHNIK JM, BURCH JA, BURCHETTE J, SHEA CR: "The SCF/KIT Pathway Play a Critical Role in the Control of Normal Human Melanocyte Homeostasis" J INVEST DERMATOL, vol. 111, no. 2, 1998, pages 233-238, XP001133837 cited in the application abstract page 237, left-hand column, paragraph 2 -page 238, left-hand column, paragraph 1 --- -/--	1-6, 9-12, 27-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2003	07/02/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Langer, O

12

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 02/04330

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 03854 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); BUERGER HANS MICHA) 28 January 1999 (1999-01-28)	29-33
Y	abstract page 3, paragraph 4 - paragraph 5 page 4, paragraph 3 formulae I and II on page 3 -page 4 page 9, paragraph 3 -page 11, paragraph 1 page 12, paragraph 2 -page 16, paragraph 3 page 17, paragraph 1 claims 10-12	1-12,27, 28

X	WO 01 47950 A (UNIV GENEVE ;IMHOF BEAT A (CH); WEHRLE HALLER BERNHARD M (CH)) 5 July 2001 (2001-07-05) page 1, paragraph 3 page 2, paragraph 4 page 3, paragraph 2 - paragraph 3 page 6, paragraph 5 -page 7, paragraph 1 page 34, paragraph 3 -page 35, paragraph 4 claims 51,60	1-6, 9-12, 27-33
Y	page 3, paragraph 2	7,8

Y	WO 00 09098 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); WOOD JEANETTE MARJ) 24 February 2000 (2000-02-24) page 12, line 5 - line 7 page 12, line 14 - line 15 table 1	7,8

X	EP 0 564 409 A (CIBA GEIGY AG) 6 October 1993 (1993-10-06) claims 1,9-11	29-33
Y	abstract page 3, line 58 -page 4, line 5 page 5, line 1 -page 5 page 5, line 39,6,49 page 16; example 21	1-12,27, 28
X	& US 5 521 184 A 28 May 1996 (1996-05-28) claims 1,23	1-12, 27-33

X	US 5 886 020 A (TANG PENG CHO ET AL) 23 March 1999 (1999-03-23) column 2, line 25 - line 37 column 8, line 50 - line 64	1-6,9-12

	-/--	

12

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/IB 02/04330

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	HACHIYA A ET AL: "The inhibitory effect of an extract of clove (Syzygium aromaticum (L.) Merr. et Perry) on ultraviolet B-induced pigmentation via inhibition of stem cell factor/c-kit signaling." JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 119, no. 1, July 2002 (2002-07), page 341 XP008012338 63rd Annual Meeting of the Society for Investigative Dermatology;Los Angeles, California, USA; May 15-18, 2002, July, 2002 ISSN: 0022-202X the whole document	1-6, 9-12, 27-33
P,X	HATTORI H ET AL: "The role of the epidermal stem cell factor (SCF)/c-kit cascade in the hyperpigmentation mechanism of lentigo senilis (LS)." PIGMENT CELL RESEARCH, vol. 15, no. Supplement 9, 2002, page 58 XP001133846 XVIII International Pigment Cell Conference;Egmond aan Zee, Netherlands; September 09-13, 2002, 2002 ISSN: 0893-5785 the whole document	1-6, 9-12, 27-33
P,X	IMOKAWA G: "Paracrine cytokine mechanisms of epidermal hyperpigmentation in UVB-melanosis, lentigo senilis and dermatofibroma." PIGMENT CELL RESEARCH, vol. 15, no. Supplement 9, 2002, page 34 XP001133844 XVIII International Pigment Cell Conference;Egmond aan Zee, Netherlands; September 09-13, 2002, 2002 ISSN: 0893-5785 the whole document	1-6, 9-12, 27-33

12

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB 02/04330

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-27 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 13-26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

International Application No. PCT/IB 02 04330

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 13-26

(A) Claims 13-26 encompass the use of a genus of compounds defined only by their function wherein the relationship between the structural features of the members of the genus and said function has not been defined. In the absence of such a relationship either disclosed in the as-filed application or recognisable by one skilled in the art based upon information readily available, the skilled artisan would not know how to make and use compounds that lack a structural definition.
The fact that one could have assayed a compound of interest using the claimed assays does not overcome this defect since one would have no knowledge beforehand as to whether or not any given compound (other than those that might be particularly disclosed in an application) would fall within the scope of what is claimed. It would require undue experimentation (be an undue burden) to randomly screen undefined compounds for the claimed activity.
Therefore no search has been performed for claims 13-26, and 27 as far as dependent from 13-26 (Article 5 and 6 PCT).

(B) Present claims 1-12, and 27-33 relate to a rather elevated number of compounds.
Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds for which a medical use is claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

(C) Present claims 1-12, and 27-33 relate to compounds/uses defined by functional characteristics, namely

(1) 'tyrosine kinase inhibitor',
(2) 'c-kit inhibitor', 'inhibitor of activated c-kit', 'c-kit inhibitor (...) capable of inhibiting SCF-activated c-kit', 'inhibitor (...) capable of inhibiting constitutively activated-mutant c-kit',
(3) 'indolinones', 'pyrimidine derivatives', 'pyrrolopyrimidine derivatives', 'quinazoline derivatives', 'quinoxaline derivatives', 'pyrazoles derivatives', 'bis monocyclic, bicyclic or heterocyclic aryl compounds', 'vinylene-azaindole derivatives', 'pyridyl quinolones derivatives', 'styryl compounds', 'styryl-substituted pyridyl compounds', 'selenoindoles', 'selenides', 'tricyclic polyhydroxylic compounds', 'benzylphosphonic acid compounds',
(4) 'N-phenyl-2-pyrimidine-amine derivatives', 'indolinone derivatives', 'pyrrol-substituted indolinones', 'monocyclic, bicyclic aryl and heteroaryl compounds',

The claims cover all compounds/uses falling under the above functional definitions, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds/uses.
Due to the choice of a functional definition of the compounds/uses of the

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

present application it is impossible to compare them with compounds/uses in the prior art for the full range of compounds/uses claimed.

(D) Present claims 1-12, and 27-33 relate to the treatment of 'melanocyte dysfunction associated diseases'.

The claims cover all medical uses falling under the above etiological definition, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such medical uses.
Due to the choice of a functional definition of the medical uses of the present application it is impossible to compare them with medical uses in the prior art for the full range of uses claimed.

(E) The desiderata in claims 2 and 9 that the tyrosine kinase inhibitor/c-kit inhibitor "is unable to promote death of IL-3 dependent cells cultured in the presence of IL-3' have been disregarded.

(F) Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely for the use of compounds of formula (II), in particular CGP57148B, for the manufacture of a medicament for whitening the human skin and for those medical uses that have been explicitly disclosed, namely hypermelanosis resulting from lentigines, solar and senile lentigo, Dubreuilh melanosis, moles and malignant melanomas and for compositions comprising tyrosine kinase compounds of formula (II), including CGP57148B, all with due regard to the inventive concept underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/IB 02/04330

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9903854 A	28-01-1999	AU	740713 B2	15-11-2001
		AU	8975998 A	10-02-1999
		BR	9810920 A	15-08-2000
		CN	1264375 T	23-08-2000
		WO	9903854 A1	28-01-1999
		EP	0998473 A1	10-05-2000
		HU	0003230 A2	28-06-2001
		JP	3276359 B2	22-04-2002
		JP	2001510192 T	31-07-2001
		NO	20000227 A	17-01-2000
		NZ	502295 A	21-12-2001
		PL	338129 A1	25-09-2000
		SK	432000 A3	12-06-2000
		TR	200000060 T2	21-09-2000
		US	2002115858 A1	22-08-2002
		ZA	9806362 A	22-01-1999
WO 0147950 A	05-07-2001	AU	2511901 A	09-07-2001
		WO	0147950 A2	05-07-2001
		EP	1240196 A2	18-09-2002
WO 0009098 A	24-02-2000	AU	5733099 A	06-03-2000
		WO	0009098 A2	24-02-2000
		EP	1105136 A2	13-06-2001
		JP	2002522475 T	23-07-2002
EP 0564409 A	06-10-1993	AT	188964 T	15-02-2000
		AU	3569493 A	07-10-1993
		BR	1100739 A3	06-06-2000
		CA	2093203 A1	04-10-1993
		CN	1077713 A ,B	27-10-1993
		CZ	9300560 A3	16-02-1994
		DE	59309931 D1	24-02-2000
		DK	564409 T3	19-06-2000
		EP	0564409 A1	06-10-1993
		ES	2142857 T3	01-05-2000
		FI	931458 A	04-10-1993
		GR	3032927 T3	31-07-2000
		HU	64050 A2	29-11-1993
		IL	105264 A	11-04-1999
		JP	2706682 B2	28-01-1998
		JP	6087834 A	29-03-1994
		KR	261366 B1	01-08-2000
		MX	9301929 A1	29-07-1994
		NO	931283 A	04-10-1993
		NZ	247299 A	26-07-1995
		PT	564409 T	30-06-2000
		RU	2125992 C1	10-02-1999
		SG	43859 A1	14-11-1997
		SK	28093 A3	06-04-1994
		US	5521184 A	28-05-1996
		ZA	9302397 A	04-10-1993
US 5886020 A	23-03-1999	US	5880141 A	09-03-1999
		US	2002102608 A1	01-08-2002
		AT	200863 T	15-05-2001
		AU	706597 B2	17-06-1999
		AU	6044196 A	30-12-1996

INTERNATIONAL SEARCH REPORT

Int ınal Application No
PCT/IB 02/04330

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5886020 A		BR 9606410 A	30-12-1997
		CA 2192797 A1	19-12-1996
		DE 29623744 U1	30-09-1999
		DE 69612649 D1	07-06-2001
		DE 69612649 T2	31-10-2001
		DK 769947 T3	13-08-2001
		EP 0769947 A1	02-05-1997
		EP 0934931 A2	11-08-1999
		ES 2159741 T3	16-10-2001
		GR 3036315 T3	31-10-2001
		HU 9701694 A2	28-06-1999
		JP 2000026412 A	25-01-2000
		JP 10504323 T	28-04-1998
		JP 3231044 B2	19-11-2001
		NO 965377 A	12-02-1997
		NZ 310109 A	28-01-1999
		PT 769947 T	31-10-2001
		WO 9640116 A1	19-12-1996
		US 6225335 B1	01-05-2001
		US 6316635 B1	13-11-2001
		US 5792783 A	11-08-1998
		US 5883116 A	16-03-1999
		US 5834504 A	10-11-1998
		US 5883113 A	16-03-1999
		US 2001027207 A1	04-10-2001

Form PCT/ISA/210 (patent family annex) (July 1992)